The Philippine Journal of Agriculture

VOL. 5 FIRST QUARTER, 1934 NO. 1

THE CULTIVATION, TOXIC CONSTITUENTS, USES, CHEMICAL ANALYSIS, AND EXTRACTION OF DERRIS

By F. T. ADRIANO

Chief, Agricultural Chemistry and Soil Technology Section, Bureau of Plant Industry, Manila

TWO PLATES

According to the reports of the American Trade Commissioner the destruction caused annually by insect pests in the United States to farm crops alone is estimated at 2,000,000,000 dollars United States currency. In the Philippines, though no exact figures are available, the value of agricultural products annually destroyed by insect pests undoubtedly also amounts to a very large sum. In fighting locusts and coco leaf miners alone the annual expenditures of the Philippine Government, not counting those of individual planters and centrals, for the last few years have exceeded 150,000 pesos a year. The fight to exterminate locusts and other major insect pests is one of the most serious agricultural problems in the Islands.

The insecticides in general use at present are chemicals; such as, arsenates, nicotine, sulphate, hydrocyanic acid, and others more or less toxic to both man and animals. There is need, therefore, of insecticides that will give maximum toxicity to insects but at the same time are harmless to man and beast.

Scientists the world over are continually searching for insecticides that are effective, cheap and not poisonous to man. In this search certain plants have been found to offer great pos-

sibilities, and some of the earliest to be discovered are the pyrethrum flowers from Japan and Yugoslavia. The Derris root, the active constituents of which were discovered by Ishikawa in Japan in 1916, is the latest find, and because of its high toxicity to insects and its harmlessness to man, the cultivation of Derris and the extraction of its active constituent, rotenone, for the manufacture of insecticides, is pregnant with commercial possibilities.

Derris(20) is the generic name of numerous leguminous climbing shrubs and vines, although some species are said to be trees. The plants are natives of the Tropics, and twelve of the forty or more species are known in the Philippines, where these plants, which possess fish and arrow poisoning properties, have various dialect names. Some of these names are *malasiag, tibalan, tibanglan, tubli, tuble, tugli, tugling pula* in Tagalog, *tubli* in Visayan and Bagobo, *upei* in Bontoc, *baot* in La Union, *lono* in Albay, *lagtang* in Samal, *tublitibauon* in Cebu, *baoet* in Zambales, and *toble-lono* in Camarines Sur.

In the Dutch East Indies, where it has been cultivated for a long time, derris is also known as "tuba root." The average annual exportation of derris root from Malaya is more than 60,000 kilos.

Although many species of derris are known, *Derris elliptica* and *Derris malaccensis* seem to be the commercial species that are commonly grown in many tropical countries, particularly in the Federated Malay States and Borneo.

TOXICOLOGY OF ROTENONE

Buckingham(5) expressed the opinion that doses of pure rotenone larger than 1 grain per pound (0.14 gram per kilo) of the body weight of a dog would be harmless when administered by mouth. Haag(14) was so convinced of the harmlessness of rotenone to his test animals—guinea pigs, rabbits, dogs, cats, pigeons, frogs, and white rats—that he swallowed 150 milligrams (equivalent to 2.3 grains) and experienced no harmful effect.

Gersdorff(12) found that a concentration of 0.075 milligram rotenone per liter (equivalent to about 1 part in 13,000,000) at 27° C. kills goldfish in two hours, which shows that rotenone is twenty-five times as toxic as potassium cyanide.

Davidson(10) found that a rotenone suspension (made by adding an acetone solution of rotenone to water) is valuable in controlling such soft-bodied insects as aphids, thrips, leaf hop-

pers, caterpillars, beetle larvæ, and mosquito larvæ, while roaches and potato and cucumber beetles resist the suspension sprays. Dust containing 1 or 2 per cent rotenone, however, was only moderately effective against soft-bodied insects.

Shepard and Campbell(21) tested the effectiveness of rotenone as a stomach poison by the leaf sandwich method and reported that it is thirty times as effective as acid lead arsenate to silkworms. Richardson and Haas(19) found that rotenone has less than one-tenth the toxicity of Paris green and sodium fluosilicate to grasshoppers. Lead arsenate, however, also has low toxicity to this insect. Campbell(6) states that against certain insects rotenone as a stomach poison is more toxic than nicotine; that against some insects it is highly effective, while against others it is ineffective; and that in general it is slower in action than the better-known insecticides.

Using different test insects, different investigators found the comparative insecticidal efficiency of rotenone, nicotine, and pyrehtrins to be as follows:

1. Davidson: (10)
   Test insect: *Aphis rumicis.*
   Findings: Rotenone fifteen times as effective as nicotine.
2. Darley: (9)
   Test insect: Sprayed upon false cabbage aphid (*Rhopalosiphum pseudobrassica* in the field and *Aphis spiraecola, marosiphum rudbeckia* on golden-rod in the laboratory.
   Findings: Rotenone about ten times as effective as nicotine.
3. Richardson: (19)
   Test insect: Greenhouse red spider mite.
   Findings: Rotenone much more toxic than nicotine.
4. Shepard: (21)
   Test insect: *Aphis rumicis* and mosquito larvæ.
   Finding: More toxic than nicotine.
5. Richardson: (19)
   Test insect: Greenhouse red spider mite.
   Findings: Rotenone slightly more toxic than pyrethrins.
6. Ginsburg: (13)
   Test insect: *Aphis pomi* De G. and *Aphis sorbi* Kalt.
   Findings: Both rotenone and derris extract are much more toxic than pyrethrin extract.
7. Yothers and Keck: (24)
   Test insect: Red spider growing on *Asparagus plumosus.*
   Findings: Sprays containing pyrethrin as the active principle were more effective than the nicotine sulphate sprays but in lesser degree than the spray containing the extract of derris root. Sprays containing a 5 per cent extract of derris were the most effective of any used.

The above tests, although made upon only a few species of insects, indicate that rotenone compares favorably with two of the most extensively used contact insecticides; namely, nicotine and pyrethrins of the pyrethrum flowers. The economic significance of this is that rotenone promises to supplement the limited supply of nicotine and, in part, at least, to take the place of pyrethrum flowers, the United States supply of which is now imported to the extent of about 10,000,000 pounds annually.

USES OF DERRIS

Various species of derris have long been used in many tropical countries as fish and arrow poisons. According to several investigators, fish killed with *Derris elliptica* can be eaten with impunity by man because fish are killed by very small quantities of the poison. It is not an antiseptic and its poisonous action is more marked on the lower than on the higher animals.

Derris is an exterminating agent for lice, fleas, and mosquitoes. It is highly effective on mosquito larvæ in concentration of 3: 1,000. For a long time it has been used by Chinese gardeners to eradicate vegetable-eating insects. Its effectiveness is known to be both as a contact and a stomach insecticide.

Various species of derris were used in different parts of the Philippines as fish poison until the passage of Act No. 1499, amended by Act No. 1685, which prohibits the use of any poisonous substance or dynamite or other explosives for killing fish.

THE TOXIC CONSTITUENTS OF DERRIS

Although different species of derris have long been used by the natives of many tropical countries for fish and arrow poisons, it was not till recently that their toxic constituents were comparatively studied. Voluminous literature(20) dealing with considerable researches in different parts of the world on the subject have thrown light on the chemistry and toxicity of several constituents of derris. The toxic constituents of derris as reported are rotenone, deguelin, tephrosin, and toxicarol. Tubatoxin, which was first reported in Japan, in 1916, by Ishikawa,(16) and is now known as rotenone, was isolated from the ethereal extract of *Derris elliptica*. Its chemical formula(20) as reported is $C_{23}H_{22}O_6$.

In alcohol solution it yields pure white crystals, variable in shape but mostly hexagonal or acicular. It is soluble in chloroform, ethylene dichloride, chlorobenzene, ether, methyl alcohol,

benzene, toluene, absolute alcohol, amyl alcohol, acetone, glycerol, petroleum ether, glacial acetic acid, and oleic acid. It is soluble in cold water, weak acids, and alkalies.

The effect of light and air on rotenone deserves special mention because of the bearing it has on the use of this material as a spray or dust for plants growing in the open. Colorless solutions of rotenone in organic solvents exposed to air and sunlight become yellow, orange, and deep red, in succession, and in some cases deposit less soluble yellow crystals which have been shown to contain dihydrorotenone and rotenone, two decomposition products that are insecticidally inert.(17) Thin deposits of dry rotenone exposed to direct sunlight outdoors also change color and lose their toxicity in the course of ten days or so. It has been found that this dry decomposition can be retarded by an admixture of lampblack to exclude the light. In diffused light the decomposition of rotenone is not detectable. One derivative of rotenone, dihydrorotenone, was found inherently more stable, yet insecticidally quite as strong as rotenone.(17) These facts seem to indicate that it is possible to stabilize rotenone or rotenone derivatives sufficiently so that they can be used under outdoor conditions for the control of chewing insects; that is, as substitutes for arsenicals.

Deguelin, $C_{23}H_{22}O_6$,(8) is a dimethoxylactone isomeric with rotenone. It is optically active as pale green platelike rods the melting point of which is 171° C.

Tephrosin, $C_{23}H_{22}O_7$ melts at 190° C. and is obtained as white, short, thick prisms.

Toxicarol, $C_{23}H_{22}O_7$, the melting point of which varies from 218 to 220° C., is obtained as greenish yellow hexagonal plates.

It will be seen, therefore, that all four compounds are closely related, rotenone and deguelin being isomeric and also tephrosin and toxicarol. Furthermore, the last two compounds differ from the former only by the presence of an additional atom of oxygen.

ANALYSIS AND METHOD OF EXTRACTION

There are few methods of analysis used by different authors with the use of various extracting liquids. Two of them, extraction with ether(20) and carbon tetrachloride, may be cited. The carbon tetrachloride method is as follows:

Fifty grams of plant material ground to about 20-mesh are completely extracted in a large Soxhlet extraction apparatus with carbon tetrachloride. The material should be extracted

from eight to ten hours (longer extracting period for samples giving more than 5 per cent rotenone). It is suggested that the extraction be run overnight (about seventeen hours), to effect a reduction in the actual working time consumed by the method. The extract is concentrated to 50 to 25 cc in a small beaker and set aside for eighteen to twenty-four hours to allow the rotenone to crystallize. The rotenone separates from the concentrated extract as needlelike crystals containing one molecule of solvent of crystallization. Just before filtering, the extract is cooled for ten to fifteen minutes on ice to assure complete crystallization. The rotenone-carbon tetrachloride solvent is filtered by suction, through a tared Gooch crucible containing a disc of hardened filter paper and washed with 10 to 20 cc of ice-cold carbon tetrachloride in small portions. As much as possible the excess solvent is removed from the precipitate by suction and then the crystalline material is dried in the crucible to constant weight in air, at room temperature (an overnight drying is sufficient). The solvent is quite stable and the loss of solvate of crystallization occurs so slowly as to be insignificant in the time mentioned. It is then weighed like the solvate, and this weight multiplied by the factor 0.719 (the ratio of $C_{23}H_{22}O_6$ to $C_{23}H_{22}O_6CCl_4$) gives the weight of rotenone.

If the amount of total extract is desired, the filtrate is freed of carbon tetrachloride by evaporation and dried for one hour at 105° C. This weight, added to the weight of rotenone, gives the total carbon tetrachloride extract (without solvent of crystallization).

The foregoing method is said to give much more selective separation of the rotenone than is possible with ether. Besides, under tropical conditions like those in the Philippine Islands, the low volatility of carbon tetrachloride makes it superior to ether for extracting the active principles of derris roots.

Samples of *Derris elliptica* produced in the Federated Malay States(11) gave the results noted in Table 1.

Dr. Valenzuela and his coworkers in the School of Pharmacy, University of the Philippines, reported that they obtained a yield of 0.1038 gram or 0.2 per cent of the purified crystals. From their analysis they claimed to have obtained rotenone.

The results of the analysis of different samples of derris root from different agricultural districts of the Bureau of Plant Industry by the use of ether for extraction are shown in Table 2.

TABLE 1.—*Yield per acre and analysis of derris elliptica roots.*

Age of roots.	Yield per acre.[a]			Ether extract.		Yield of ether extract per acre.		
	Fresh roots.	Moisture free.		Fine roots.	Coarse roots.	Fine roots.	Coarse roots.	Total roots.
		Fine roots.	Coarse roots.[c]					
Mos.	*lbs.*[b]	*lbs.*	*lbs.*	*P. ct.*	*P. ct.*	*lbs.*	*lbs.*	*lbs.*
21	1,277	240	211	7.88	6.99	18.9	14.7	33.6
23	2,190	403	201	9.63	4.52	38.8	9.1	47.9
25	2,697	576	149	8.59	3.18	49.5	4.7	54.2
27	3,445	751	317	5.17	4.84	38.8	15.3	54.1

[a] 1 hectare=2.471 acres.

[b] 1 kilo=2.2 lbs.

[c] Coarse roots are those thicker than an ordinary pencil.

TABLE 2.—*Analysis of different samples of derris.*[a]

Sample No.	Origin.	Local or common names.	Size of roots analyzed.	Results of analysis.				
				Moisture.	Ether extract.		Crude rotenone.	
					Dried at 70° C.	Moisture free.	Dried at 70° C.	Moisture free.
					P. ct.	*P. ct.*	*P. ct.*	*P. ct.*
Ac–33–81	Calapan, Mindoro; delivered by Mr. Aguanta; growing wild; flowers and pods available during August.	Tubli	Small [b]	8.62	7.85	8.59	0.05	0.05
Ac–33–81	do	do	Medium [b]	15.04	7.23	8.51	0.03	0.04
Ac–33–81	do	do	Big [b]	12.03	6.55	7.45	0.02	0.02
Ac–33–84	Brownish in color, from forest of municipal district of Alilem, Ilocos Sur; planted in backyard of Mr. Navalta; creeping and climbing; wild flowers in November and December.	Ba-ot	Medium	6.84	5.94	6.38	0.08	0.09
Ac–33– 84	Whitish in color; taken along brook of forest in Puspus, San Fernando, La Union, 12 km. from the town; said to flower in November and December; both from Mr. Albayalde.	do	Big and medium	7.96	5.79	6.29	0.21	0.23
Ac–33–102	From Lamao Experiment Station, Bataan; no information given.		Medium	5.73				
Ac–33–103	Sent by Mr. Domingo Baybay from Guinobatan, Albay; no information given.	Lono	Small and medium	29.92	0.30	4.64	0.03	0.04
Ac–33–109	Sent by Mr. Medrano, delivered by Mr. Lontok, from Iba, Silang, Cavite; cultivated but only few plants to an owner.	Tubli	Mixture of medium and small.	5.51	5.37	5.68	0.09	0.10
Ac–33–103	Sent by Mr. Domingo Baybay, Guinobatan, Albay; no information was given.	Lono	Medium and small	7.56	12.76	13.80	0.39	0.42

Ac-33-110	Sent by Mr. Medrano, delivered by Mr. Lontok; Pulongbuñga, Silang, Cavite; cultivated but few plants to an owner.	Tubli	Medium	6.97	2.65	2.85	0.06	0.06
Ac-33-112	Sent by Mr. Medrano, delivered by Mr. Lontok; no information is given for no tag was found.	do	Medium and small	7.70	4.80	5.20	0.15	0.16
Ac-33-111	Sent by Mr. Medrano, delivered by Mr. Lontok; Paliparan, Dasmariñas, Cavite; cultivated but only few plants to an owner.	do	Big and medium	6.31	3.22	3.44	0.04	0.04
Ac-33-132	From Mr. Antonio Llanes, Maquiling, Calamba, Laguna; creeping and climbing, some growing wild while others are planted along fences.	do	Medium and small	2.94	5.51	5.68	0.15	0.15
Ac-33-136	From Pacifico Marcelo, San Rafael, Camarines Sur; creeping, 2 or 3 plants cultivated by a family near river and creek and dig roots in March and April.	Toble-lono	Medium	3.24	4.47	4.62	0.57	0.59
Ac-33-129	From Mr. Rosendo, Ventura, Occidental Misamis; no information received.		do	3.71	12.64	13.13	1.63	1.68
Ac-33-135	Sent by Mr. Primo Santiago, Oriental Misamis; no information was given.		do	3.21	9.39	9.70	0.79	0.82
Ac-33-108	Sent by Mr. Medrano, delivered by Mr. Lontok; Lalaan, Silang, Cavite; cultivated but only few plants to an owner.	Tubli	do	2.41	15.85	16.24	0.36	0.37
Ac-33-116	Origin is Samal, Davao; sent by Mr. Bonifacio Padilla; climbing and wild.	do	do	4.06	4.23	4.41	0.05	0.05
Ac-33-134	Sent by Mr. Primo Santiago, Cagayan, Oriental Misamis; no information.	Doles	do	5.47	4.16	4.40	0.09	0.10
Ac-33-115	Lagtang, Talisay, Cebu; from Mr. Antonio Derecho; creeping and climbing, about 300 kilos per hectare.	Ikog-ilaga	do	6.62	17.68	18.93	0.89	0.85
Ac-33-114	Lagtang, Talisay, Cebu; from Mr. Antonio Derecho; creeping and climbing; semiwild.	Tibauon	do	2.80	3.78	3.89	0.21	0.22

[a] Samples for identification sent to the Bureau of Science.
[b] Less than 1 cm in diameter.
[c] From 1 to 1.5 cm in diameter.
[d] From 1.5 to 2.5 cm in diameter.

TABLE 3.—*Analysis of some varieties of derris roots.*

Varieties of derris.	Nature of roots.	Crude rotenone.	Total ether extract.	Proportion of crude rotenone to total ether extract.
		P. ct.	*P. ct.*	*P. ct.*
Derris elliptica: tuba puteh	Coarse	6.5	22.6	28.8
	Fine	6.8	24.8	27.4
Derris elliptica; creeping, Sarawak	Coarse	No.	Coarse.	Roots.
	Fine	Trace.	4.0	----------
Derris malaccensis; tuba merah	Coarse	0.7	14.5	4.8
	Fine	0.1	19.8	0.5
Derris malaccensis; erect, Sarawak	Coarse	0.1	24.2	1.7
	Fine	0.8	27.3	2.9
Derris polyantha	Coarse	2.7	12.6	21.4
	Fine	3.6	14.4	25.0
Derris (species not identified)	Coarse	Nil.	3.0	----------
	Fine	Nil.	2.3	----------

PLANTING OF DERRIS

Although many species of derris are known, only a few are considered of any commercial value, probably because of their ease of cultivation and propagation and their higher rotenone content. *Derris elliptica* and *Derris malaccensis* seem to be those most commonly grown for export.

Derris is easily propagated by means of stem cuttings about 8 to 20 inches long. The cuttings are usually planted in sandy soil. The leaves should be removed if the planting is done during sunny weather. It is often better to plant them in slightly shaded nurseries to root. After about six weeks they develop enough roots for transplanting.

The cuttings are usually planted in rows 3 feet apart spaced 3 feet apart in the rows. This planting arrangement will give about 4,840 plants per acre or about 12,000 plants per hectare. In the Federated Malay States derris is usually interplanted with kapok and chilies. The kapok trees are spaced 20 by 20 feet square. With this planting arrangement there are 3,555 plants to the acre or 8,784 plants to the hectare.

In the Philippines, particularly in Cebu, some farmers plant derris on a small scale. As far as can be ascertained, the largest derris plantation is in the Calamba Sugar Estate at Canlubang, Laguna.

HARVESTING

According to the experiments conducted in the Federated Malay States (2) on *Derris elliptica,* the roots should be har-

vested between twenty-one and twenty-three months after planting, since a definite reduction in the amount of either extract is noted when the roots are allowed to remain for twenty-five months before harvesting.

The yield of commercial roots has been calculated at about 2 tons per hectare.

DEALERS IN AND CURRENT PRICES OF DERRIS

The reports on file in the office of Mr. E. D. Hester, American Trade Commissioner, contain the following list of prospective United States purchasers of derris roots:

1. Kay Laboratories, Inc., 180 Madison Avenue, New York City.
2. Geo. H. Lincks, 123 Front Street, New York City.
3. United Chemical and Drug Corporation, 135 Williams Street, New York City.
4. J. L. Hopkins and Co., 135 Williams Street, New York City.
5. S. P. Penick and Co., 115 Fulton Street, New York City.
6. McCormick and Co., Inc., 400 Light Street, Baltimore, Md.
7. McLaughlin, Gormley and Kink Co., Minneapolis, Minn.
8. John Powell and Co., 114 East 32d Street, New York City.

According to quotations furnished by the Manila representative of Getzbest Bros., November, 1933, dry derris roots are sold in New York City at about 50 centavos per pound.

THE PREPARATION OF DERRIS ROOTS FOR THE MARKET

When the cultivated plants are about 21 to 23 months old they are ready for harvest. The harvested fine roots are washed free from soil and other adhering dirt with water. In Malaya the dried roots are pressed into bales and then wrapped with jute bags and exported in this form to the United States, Japan, and Europe.

Sometimes, in order to reduce the bulk of material for ocean shipment, the dried roots are chopped and ground into a coarse powder and baled or packed in tin cans.

It has been found that the toxic constituent of the rotenone content of the most potent dry roots is about 7 per cent, so that 93 per cent is inert material. As much as possible of it should be eliminated in preparing derris for exportation. Drying in the sun or other methods of artificial desiccation may result in some loss of toxic constituent. However, if the marketing of derris is to be undertaken on a large scale, and it should be in order to make it pay, the use of vacuum driers and extraction

with volatile solvents such as ethyl alcohol, benzine, carbon tetrachloride, and ethylene dichloride offers commercial possibilities. The solvents that might be of practical use are benzene, alcohol, and ethylene dichloride. The derris extract can be concentrated under vacuum to a thick syrup and the solvent recovered. The concentrated extract can be diluted to any desired concentration.

It would seem necessary that the sale of derris in whatever form should be regulated by analysis, since it has been found that different varieties of roots have a highly variable percentage of rotenone.

LITERATURE CITED

1. Anonymous. British Empire Exhibition, London. Malayan ser. 8 (1924) 46 pp. Miscellaneous crops.
2. Anonymous. Recent research on Empire products. Miscellaneous agricultural products. Bull. Impt. Inst. (London) 27 (1929) 507. Cited by Roark.
3. Bacon, R. Fish poison used by the natives. Philip. Journ. Sci. 1 (1906) 1025–1029.
4. Boehinger, C. A. 1930. Derris (tuba) root production in Straits Settlements. U. S. Dept. Com. Bur. Foreign and Dom. Com. World Trade Notes on Chemicals and Allied Products. (4) 5 (1930) 11–12.
5. Buckingham, D. E. Ind. Eng. Chem. 22 (1930) 1133.
6. Campbell, F. L. Dept. Agr. Bur. Entomol. Mimeographed Circ. E–298 (1932).
7. Castillo, N. Preliminary studies on the insecticidal properties of three species of derris in the P. I. Philip. Agriculturist 15 (1926) 257–275.
8. Clark, E. P. Some constituents of derris and cube roots other than rotenone. Science (n. s.) 71 (1930) 396. Cited by Roark.
9. Darley, M. M. Journ. Econ. Ent. 24 (1931) 111.
10. Davidson, W. M. Journ. Econ. Ent. 23 (1930) 969.
11. Georgi, C. D. V., and E. A. Cutter, 1929. The periodic harvesting of tuba root. (Derris elliptica Benth.). Malayan Agr. Journ. 17 (1929) 326–334.
12. Gersdorff, W. A. Journ. Am. Chem. Soc. 52 (1930) 3440.
13. Ginsburg, J. M. Journ. Econ. Ent. 25 (1932) 1237–40.
14. Haag, H. B. Journ. Pharmacol. Exptl. Ther. 43 (1931) 193.
15. Hester, E. D. Reports of the American Trade Commissioner, Manila (1933).
16. Ishikawa, T. Investigation of tuba, an East Indian poison for fish. Tokyo Igakkawai Zasshi, 30 (1916) 45–46. Japan Med. Lit. 1 (pt. 2): 7–8. Cited by Roark.
17. Jones, H. A., et al. Journ. Am. Chem. Soc. 53 (1931) 2320.
18. La Forge, F. B., et al. Chem. Rev. 12 (1933) 181.
19. Richardson, C. H., and L. E. Haas. Journ. Econ. Ent. 23 (1932) 1078.

20. ROARK, R. C. A digest of the literature of *Derris deguelia* species used as insecticides. 1747–1931. United States Dept. Agr. Miscellaneous Publication, No. 120 (1932).
21. SHEPHARD and CAMPBELL. Journ. Econ. Ent. 25 (1932) 142–44.
22. SPOON, W., Jr. Verdere waarnemingen over de samenstelling van Derris-wortel of Akar toeba. De Indischen Mercur 55 No. 13 (1932) 181–183.
23. UICHANCO, V. B. A study of three species of Philippine Derris. Master's dissertation School of Pharmacy, University of the Philippines (1933) 136 pp.
24. YOTHERS, W. W., and C. B. KICK. Sonthom Florist Nurseryman 29 (1930) 7.

ILLUSTRATIONS

Plate 1

Fig. 1. Some derris plants (Derris elliptica) grown at the Bureau of Plant Industry Central Experimental Station, Manila.

2. A closer view of the derris plant shown in fig. 1.

Plate 2

Fig. 1. Samples of derris roots collected from different plants of the Philippines which have been analyzed for rotenone. Bottles contain derris root powder. Arrow points to a tube containing rotenone which has been extracted from derris roots. Agricultural Chemistry and Soil Technology Laboratories, Bureau of Plant Industry, Manila.

2. Derris-root grinder, in the agricultural chemistry and soil technology laboratories, Bureau of Plant Industry, Manila. (Photograph by the Division of Publications.)

1

2

PLATE 1.

1

2

PLATE 2.

THE EFFECT OF MULCHING ON THE YIELD OF EXCELSA COFFEE: A PROGRESS REPORT FOR 1930–1933

By F. D. Luistro

Acting Station Superintendent, Bureau of Plant Industry, Manila

and

Gaudencio Evaristo

Assistant Agronomist, Bureau of Plant Industry, Manila

The favorable effects of mulching permanent crops during the dry season are well recognized in scientific agriculture. Mulch keeps down the weeds and conserves the moisture and fertility of the soil. It loosens the soil, and thus improves its aëration and water-holding capacity. In the Philippines, however, mulching has been studied very little, especially in connection with Excelsa coffee. To determine the effect of mulching on the yield of this crop, this experiment was conducted at the Maridagao Rubber Station, Pikit, Cotabato, Mindanao.

MATERIALS AND METHODS

Level ground of practically uniform fertility, covering an area of 6,804 square meters located on the western side of the station along Maridagao River, was used in this test. The field was second-growth forest, and before any planting was done, it was prepared and cleaned as uniformly as possible.

The field was divided into eight equal plots, and each plot planted to forty-two seedlings of Excelsa coffee 4.5 meters by 4.5 meters apart on June 5, 1930, under ipil-ipil shade with Calopogonium-Centrosema cover crops. The planting was made in four replications as indicated in the following:

Mulched plots.	Control plots.
I	II
III	IV
V	VI
VII	VIII

Mulching materials composed of Calopogonium-Centrosema vines, of 10 kilos, more or less, for each tree, were applied at

the close of the rainy season and the portion left undecomposed removed at the end of the dry season. The trees in the control plots were merely kept ring-weeded. Other cultural treatment in regard to drainage, pruning, and eradication of pests and diseases, were as uniform as possible.

Field observations were made on the general growth of the plants and their susceptibility to diseases and pests. The crops were harvested as they matured, and the weight of fresh berries from the different plots recorded.

RESULTS AND DISCUSSION

TABLE 1.—*The plots mulched, the dates of mulching and the dates when the undecomposed portion of the mulching materials were removed from the trees.*

Plot No.	Dates mulched.	Undecomposed mulching materials removed.
I, III, V, and VII	Each plot was mulched December 2, 1931, December 12, 1932, and November 6, 1933.	April 12, 1932, and May 7, 1933.

Observations made at the end of 1931 showed that there was no marked contrast in the general appearance of the treated and the non-treated trees, for mulching had been applied too recently. December 21, 1932, the treated trees were found excelling the control trees in height, spread of branches and in fruiting habit, obviously being more resistant to drought than trees that were not mulched.

TABLE 2.—*The yield of the mulched trees in fresh berries by plots during the year.*

Plot No.	Trees.	Yield.	Remarks.
		Kilos	
I	42	0.970	Harvesting not yet finished, plenty still on the trees.
III	42	2.315	
V	42	2.950	
VII	42	10.840	
Total	168	17.075	

TABLE 3.—*The yield of the unmulched trees in fresh berries by plots during the year.*

Plot No.	Trees.	Yield.	Remarks.
		Kilos	
II	42	2.490	Harvesting not yet finished, plenty still in the trees.
IV	42	1.320	
VI	42	2.770	
VIII	42	2.510	
Total	168	9.09	

From tables 2 and 3 it appears that in the first replication the control trees gave more than the treated ones; that is, 2.490 kilos from the unmulched trees as against 0.970 kilo from the treated trees. This cannot be taken conclusively, as harvesting of this year's crop is not yet finished, for there are plenty of green berries still in the trees. From ocular inspection the treated trees seem to have more fruits than the controls, and most likely, when harvesting is over, the yield of the treated trees in the first replication will be more than that of the control trees. Taking the total yields as the bases of comparison, the treated trees yielded more than the control trees by 87.8 per cent, which shows conclusively the beneficial effects of mulching Excelsa coffee.

SUMMARY

The effect of mulching on the general appearance of Excelsa coffee trees was marked one year after application. The treated trees were found excelling the control trees in height, spread of branches, and in fruiting habit.

Mulching increased the yield of Excelsa coffee by 87.8 per cent, conclusively showing its beneficial effects to the crop.

GERMINATION OF THE POLLEN OF RICE AND POLLENTUBE GROWTH [1]

By GAUDENCIO M. REYES

Of the Bureau of Plant Industry, Manila

ONE PLATE

Studies on pollen-tube growth and behavior in culture have been undertaken rather extensively in other countries with various plants, but little or no work of this kind has been done in the Philippines. There seems, however, not much significance in germinating artificially the pollen of rice, or of other plants, except to determine the percentage of germination and to compare the viability in different varieties; this study was undertaken, therefore, not so much for its economic, as for its scientific interest. The work herein reported was undertaken incidentally in connection with a phase of investigation on the occurrence of a form of sterility in many rice varieties.

The first attempt in the Philippines to germinate rice pollen artificially with the use of various concentrations of cane sugar (97 per cent purity) produced negative results. (3) It was observed that the pollen grains burst in sugar solutions of from 1 to 25 per cent. Similar results were obtained in sowings made in water and in solutions of over 45 per cent concentration. There seems to be no available literature on work conducted along this line in other countries. The present paper presents the results of experiments carried on at the Alabang Rice Experiment Station of the Bureau of Plant Industry to induce the development of the pollen tubes of rice in artificial cultures. All tests were made on the veranda of the station's laboratory, which was practically an open space, with the temperature ranging from 27° to 30° C. At the time of the experiment rice plants were in bloom near-by, from which fresh pollen was readily available for this study.

THE POLLEN OF RICE

It would not probably be out of place to describe here in some detail the characteristic morphological features of the

[1] This study was made while the author was connected with the Bureau of Science, Manila.

pollen of rice, before recounting the results of the germination trials. The anthers of the rice flower dehisce usually through two openings, bigger at the tip, on each lateral side, and the pollen which they shed resembles powdered sulphur. The mature, normal pollen grains are variable in size but fairly round and smooth and regular in shape. Under the microscope they exhibit a seemingly granular content having a yellowish tint, surrounded by two thin, elastic membranes (exine and intine), (Plate 1, fig. 1). When the pollen grains are pressed lightly under the cover-glass, the translucent, partially starchy contents come out (fig. 2, a). Stained with iodine solution, they are rendered very conspicuous, being of a dark blue color, generally rodlike or oblong in shape, the ends being generally blunt, although some are almost round and others have flat ends. Sometimes they are dented slightly on one side like a kidney bean; others are slightly constricted at the middle, like a two-seeded peanut pod; still others are bulging at one end. They vary greatly in length and in width (fig. 2, b), some being long and narrow, or vice versa, and some long and broad, or vice versa. In length they vary from 1.33 to 6.11 microns and in width from 1.33 to 2.66 microns.

When the contents are extruded, the germ pore (fig. 3) is very conspicuous and has a diameter of about 3.99 microns. The surrounding cytoplasm is also distinguishable under the high power of a microscope. Water mounts of the normal, fresh pollen grains obtained from the flowers of Guinangang Str. 1 variety, were found to vary from 36.05 to 57.0 microns in diameter.

HANGING-DROP CULTURES OF THE POLLEN GRAINS

METHOD AND MATERIALS

The method followed, with a slight modification, was that of Popenoe. (2) All materials used were previously sterilized and precautions were taken to avoid contaminations. In other words the preparations were done expeditiously, avoiding unnecessary handling. On a glass slide was mounted a small glass ring, about 1 centimeter in height and 1.6 centimeters inside diameter, the cut ends of which were previously covered with sterilized vaseline. On a cover glass a platinum loopful, or two (droplets), of the sterilized culture medium was placed in the center. With the help of sterile forceps pollen grains from a dehiscing anther were placed upon the medium. The cover-glass

was then inverted and pressed carefully over the upper end of the ring, also greased with vaseline, until no air passages could be found. The hanging-drop culture was then immediately examined under the microscope, properly labelled, and placed inside a large Petri dish (15 cm diameter and 2 cm thick). The improvised glass rings served the purpose admirably.

Most sowings were done in the morning in order to simulate the habit of the plant when pollination generally takes place under favorable weather, and in order to obtain pollen in its most viable stage. Various concentrations of refined sugar, rice water, agar-sugar mixture, nectar secreted sufficiently by flowers of certain common ornamentals having tubular corolla, collected early in the morning, and sterile water with a stigma of rice flower, were tested, and the results of these preliminary tests are given in Table 1. To test the sterility of the media they were not used until a week after preparation.

TABLE 1.—*Results of trials in germinating rice pollen in hanging-drop cultures.*

Date set.	Condition of flower and position of anther.	Medium used.	Time of sowing.		Result.	Remarks.
			a. m.	*p. m.*		
November 1, 1932	Anthers extended inside the glumes.	0.6 per cent agar-agar plus 0.6 per cent sugar.	9.39		No germination	Almost all pollen grains burst.
Do	do	Sterile water		1.50	do	Contents disturbed; majority burst later.
Do	do	0.6 per cent sugar solution		2.15	do	Swelling followed by bursting.
November 2, 1932	do	Nectar of violeta [a]	8.56		Few initial growths	Nearly one-half burst.
Do	do	Nectar of santán [b]	9.20		Incipient appearance of tube at the outer wall.	Very few burst.
Do	Tip of glumes beginning to separate.	Nectar of *Pseuderanthemum* sp.[c]	9.58		No germination	None burst.
Do	Flower about to open, anthers extended inside the glumes.	Nectar of violeta with a mature rice stigma.	10.25		Initial signs of tube growth.	Very few burst.
November 3, 1932	do	Sterile water with a mature rice stigma.	10.40		No germination	Nearly all burst.
Do	do	Dilute rice water	11.35		Few showed signs of germination.	Few swelled and somewhat plasmolyzed.
November 4, 1932	do	do	8.27		Few germinated	Few burst and few somewhat plasmolyzed.
Do	do	Rice decoction, medium concentration with a mature rice stigma.	9.45		Very few showed signs of germination.	Few did not burst but many were plasmolyzed.
Do	do	Rice decoction, concentrated solution	9.55		No germination	Nearly all burst; those not burst were plasmolyzed.
Do	do	0.8 per cent agar plus 0.8 per sugar.	10.10		do	Do.

Do	do	1 per cent agar plus 1 per cent sugar.	10.25	do	Majority burst or plasmolyzed.
Do	do	Nectar of violeta	10.35	do	No bursting but with slight signs of plasmolysis.
Do	do	10 per cent sugar solution	11.15	One showed initial sign of germination.	Few burst; many plasmolyzed.
Do	do	Concentrated rice water	11.25	Very few showed initial signs of tube growth.	Many plasmolyzed and few burst.
November 8, 1932	do	3 per cent sugar solution	8.35	No germination	Very few burst.
Do	do	5 per cent sugar solution	8.45	None germinated	Few burst; many plasmolyzed.
Do	do	1 per cent sugar solution	8.50	do	Many burst.
Do	do	10 per cent sugar solution	9.20	do	Many plasmolyzed; few burst.
Do	Anthers out of glumes; flower open.	0.6 per cent agar plus 0.6 per cent sugar.	9.50	Few initial appearance of end of hyaline tube.	Germinating pollen burst.
Do	do	Sterile water	10.09	No germination	Nearly all burst.

[a] Violeta (Spanish); *Barleria cristata* Linn.

[b] Santán (Tagalog); a vernacular name for *Ixora finlaysoniana* Wall.

[c] *Pseuderanthemum* sp.; possibly *P. reticulatum* Radlk. (1)

RESULTS

It was found that the rice pollen is quite difficult to germinate. The little success so far obtained seemed to be contingent upon certain factors as follows: (*a*) The immediate use of fresh material right in the field; (*b*) the selection of the right stage of the flower, i. e., when the panicle is normal in every respects, having the yellow anthers nearly ripe or fully extended within the enclosing glumes which could be readily seen by holding the florets against the light; (*c*) use of the right medium; and (*d*) strict observance of all the necessary aseptic precautions. Under Alabang conditions the most viable pollen grains were obtained from normal plants, and flowers which were about to open between 8 and 11 in the morning in a bright day. Anthers plucked from such flowers, when forced open, dehisce almost instantly and the pollen grains are liberated and fall freely of their own weight, thus greatly facilitating their sowing on any culture medium.

DISCUSSION

Present results of hanging-drop cultures seem to indicate that dilute rice decoction having a pH value of 6.5 (using Merck's universalindikator) is the most successful medium (figs. 4 and 5) although initial growths were also obtained on rice water of medium concentration and on the sweet nectar of violeta (*Barleria cristata* Linn.) (1) Sowings made in the morning gave better results than those made in other parts of the day; since rice pollination usually takes place in nature in the morning it is probable that the pollen grains are in their most suitable condition for germination in the morning. The percentage of germination, however, was in every case low. On dilute rice decoction incipient growths were observed in about one-fourth of a day, and the germination was very feeble, ceasing in three days (fig. 5). The longest pollen tube observed measured 14.25 μ. There were many others much longer, but they could not be measured with any degree of accuracy because generally the tubes were either twisted or curved (fig. 5). Only one tube was observed protruding from each pollen grain. It seems however that the pollen tube would not grow very long as it has only a short distance to travel in the style.

Bursting of the pollen grains was observed in varying numbers in sterile water, sugar solutions, agar-agar-sugar media of different concentrations (fig. 6), and starch solutions of high concentrations or else the grains were either distended or

plasmolyzed (fig. 7), vacuoles appearing in some in the protoplasm. In dilute starch solutions much fewer cases of bursting were noticed, while in nectar the grains remained almost all normal at times. There is a disadvantage in using fresh nectar, however, in that fungous contaminations are likely to occur and these may interfere with the normal development of the pollen tube. It is believed that if the concentration and H-ion reaction of the sticky secretion (stigmatic fluid) in the stigma could only be determined, or if its chemical constituent could be analyzed so that it could be imitated, the germination of the pollen of rice could be accomplished much more easily.

The protrusion of the tube took place around the membrane surrounding the germ pore (fig. 8). It was further observed under the microscope that the content of the pollen ran out into the pollen tube in a very slow motion, probably due to the contraction of the inner wall (intine) the contents passing through the germination pore. The contents which are rod-shaped sometimes aligned themselves somewhat in concentric fashion and flowed out slowly together with the cell sap which served as vehicle.

In newly pollinated stigmas this movement of the pollen content could be detected more distinctly, the pollen becoming nearly or entirely empty, and the pollen tube could be seen penatrating the papillae or plumose tissue of the stigma (fig. 8).

The percentage of germination and the rapidity of tube development were very much inferior to those of the pollen of other flowers, especially that of santán (*Ixora finlaysoniana* Wall.) (1) which germinated readily in water (or sugar solutions of the right concentration), the pollen tube reaching a length of 176.7 μ in three hours and forty minutes. While the germination of pollen is comparatively rapid in most plants, although not as fast as in some fungous hyphae, the germination of rice pollen is an exception. It should be borne in mind, however, that the pollen tube has a wide range of growth in different plants.

SUMMARY

An examination of the pollen grains of normal rice was made and a description is here given in some detail.

Pollen grains of rice are in their most viable stage generally about nine and ten o'clock in the morning. Sowings made in the morning in artificial media gave better results than those done in other times of the day.

Tests were made of pollen-tube growth in various culture media of different concentrations and on its reaction to the secretions of the stigma. In almost all cases varying numbers of distention and bursting, or plasmolysis and bursting occurred. In dilute starch solutions and nectar more pollen grains were observed to remain normal than in other culture media.

It was found that the pollen tubes of rice grew at a very slow rate, if induced artificially, as compared with those of other plants, the longest measurable growth observed being only 14.25 μ in dilute rice decoction having a pH value of 6.5.

Only one tube has been observed thus far to protrude from each pollen grain.

The pollen grains responded readily in the presence of the stigma, but when a stigma was placed in a droplet of sterile water, rice decoction, or nectar sown with rice pollen, thereby causing a dilution of the stigmatic fluid, only a slight noticeable influence was seen.

The other essential factors which seem to contribute to the successful production of pollen tube are the use of fresh material, the selection of the right stage of the flower, and absolute adherence to all necessary aseptic precautions.

The writer desires to express his appreciation to Mr. Juan O. Unite, acting superintendent of Alabang Rice Experiment Station, Bureau of Plant Industry, for his many courtesies.

LITERATURE CITED

1. Merrill, E. D. Enumeration of Philippine Flowering Plants 3 (1923) 479, 483, 548.
2. Popenoe, Wilson. The pollination of the mango. U. S. Dept. Agr. Bull. 542 (1917) 1–20.
3. Rodrigo, P. A. Pollination and the flower of rice. Philip. Agrst. 14 (1925) 155–171.

ILLUSTRATIONS

Plate 1

Fig. 1. Normal pollen grains of rice drawn under the high power of a microscope as seen through transmitted light; × 303.

2. *a*. Normal pollen grains pressed lightly under a cover glass to show some of their rod-shaped contents and the germ pore as seen under a high-power microscope; × 303.

b. Contents of pollen drawn under immersion lens, to show various sizes and shapes; × 612.

3. Highly magnified drawings of the germ pore as viewed in two positions under an immersion lens to show the germination pore, the translucent membrane, and the surrounding cytoplasm; × 612.

4. Rice pollen showing the development of the pollen tube in rice decoction; × 240.

5. Pollen sown in dilute rice decoction showing distorted development of the pollen tubes; × 240.

6. Burst pollen grains observed in 0.6 per cent agar-sugar hanging-drop cultures with some of the contents extruded; × 240.

7. Plasmolyzed pollen grains observed in sowings made in rice decoction of medium concentration, preceded by swelling; × 240.

8. Germinating pollen grains obtained from pollinated flower showing from where the tubes originate and how they penetrate the plume-like stigmatic branches. Note that part of the pollen content has been emptied into the pollen tube; × 240.

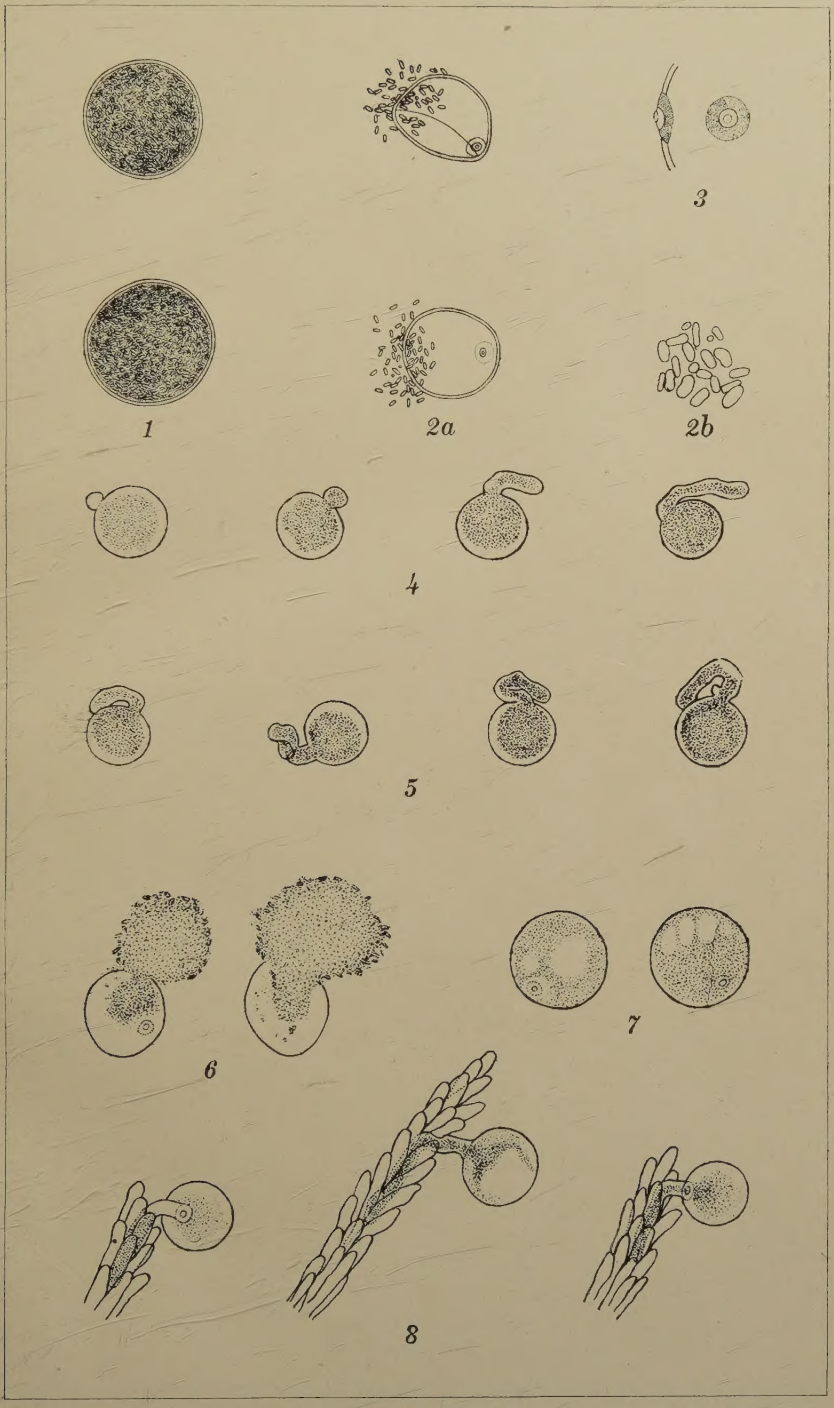

PLATE 1.